

Role of Natural Compounds in Prevention and Treatment of Chronic Lymphocytic Leukemia

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1 INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a malignancy of CD5⁺ B cells that is characterized by the accumulation of small, mature-appearing neoplastic lymphocytes in the blood, marrow, and secondary lymphoid tissues. Genetic factors contribute to the development of CLL. CLL is more common in western countries relative to Asian countries [1]. CLL can be divided into two main subsets. These subsets are distinguished by express a mutated or unmutated immunoglobulin heavy-chain variable region gene (IGHV). Patients with unmutated IGHV CLL cells typically have more aggressive disease than patients with CLL cells that express a mutated IGHV [2]. Several studies have disclosed numerous genetic alterations in CLL, including chromosomal alterations, single-nucleotide polymorphisms (SNPs) and alterations in noncoding RNA [3,4]. The average incidence of CLL is different in geographical regions and ranges from <0.01% in Asia to ~0.06% in Europe and the United States. The risk of CLL is about two times higher for men than for women and increases with age [5]. Genetic factors contribute to CLL susceptibility. Patients with CLL have an 8.5-fold increased risk of developing this disease, and the conformity of CLL is higher among monozygotic twins than among dizygotic twins. SNPs are associated with familial CLL; they are the most common genetic variation in this patients that contributes to heritable risk [6,7].

Reports have shown that exposure to Agent Orange is an environmental risk factor for CLL. In addition, studies suggest that exposure to insecticides might also be a risk factor for this disease. Also, little evidence has

demonstrated that ionizing radiation can increase the risk of CLL. In addition, there is little evidence that viral infections are risk factors, and epidemiological studies have not found evidence that blood transfusions can transmit CLL. No studies showed that lifestyle and dietary factors increase the risk of CLL [8].

Nearly 80% of cases with CLL convey at least one of four common chromosomal alterations: a deletion in chromosome del(13q), del(11q), del(17p) and trisomy 12. Del(13q) is the most common chromosomal alteration. Within this deleted region is a part that controls the expression of proteins that can inhibit apoptosis or that are involved in cell cycle progression [9].

The landscape of CLL has undergone deep changes in the recent years. CD20-targeting antibodies have improved for conventional chemotherapy and have shown a therapeutic outcome in the majority of CLL patients. Due to the establishment of the critical role of the B cell receptor signaling pathway in the pathogenesis of CLL, several agents have been developed to target this pathway. Lately, the observation of high expression levels of the antiapoptotic mitochondrial protein Bcl-2 in CLL has led to the development of new compounds that inhibit Bcl-2 and has shown high efficacy in CLL [10].

For over 40 years, natural products have been used in cancer therapy. The main sources of products with anti-cancer activity are plants from the marine and terrestrial environments. A major contribution comes from plants such as alkaloids, taxoids, and podophyllotoxins. Due to the rapid development of resistance to chemotherapeutic drugs, the search for novel drugs is still a priority goal for treatment of CLL. Moreover, the high toxicity usually

associated with some cancer chemotherapy drugs and their undesirable side-effects increase the demand for novel anticancer drugs active against untreatable cancer such as CLL, with fewer side-effects and with higher therapeutic efficiency [11].

2 CHRONIC LYMPHOCYTIC LEUKEMIA

Chronic lymphocytic leukemia (CLL) is a malignancy of CD5⁺ B cells that is distinguished by the cumulating of small, neoplastic lymphocytes in the blood, marrow, and secondary lymphoid tissues, leading to lymphadenopathy, leukemia cell infiltration of the marrow, lymphocytosis, and splenomegaly. Previous studies showed that genetic factors help in the development of CLL and it is the most common adult leukemia in Western countries compared with Asia. It is relatively rare in Korea and Japan, even among Japanese people who migrate to Western countries [1]. CLL is divided into two main subtypes, which are difference in clinical behavior. These subtypes are determined by whether CLL cells express a mutated or unmutated immunoglobulin heavy chain variable region gene (IGHV) [12]. CLL was estimated to account for 19,000 new cases in the United States in 2016. The average incidence of CLL varies between individuals in different geographical regions. The risk of CLL is about two-fold higher for men than for women and increases with age [5]. Genetic factors help in disease susceptibility. Relatives of patients with CLL have an 8.5-fold increased risk of developing this disease, and the concordance of CLL is higher in monozygotic twins compared with dizygotic twins. Genome-wide association studies have confirmed single nucleotide polymorphisms (SNPs) in nearly 30 loci that are related with familial CLL, demonstrating that common genetic variation contributes to heritable risk. The altered expression of genes in this location might contribute to disease development [13]. Several studies have showed that exposure to Agent Orange is a risk factor for CLL [14]. Moreover, evidence suggests that exposure to insecticides might be a risk factor as well [15]. Also, little evidence shows that ionizing radiation can increase the risk of CLL [16]. In addition, there is little investigation that viral infections are risk factors, and studies have not found evidence that blood transfusions can transmit CLL. No evidence suggests that lifestyle factors or dietary factors increase the risk of CLL [17].

3 NOVEL TARGETS FOR CLL THERAPY

The clinical course of CLL is highly variable, but most patients will eventually require treatment and indications for treatment are related to the clinical stage. Treatment

options for the management of CLL have evolved significantly in the past decades. The initial chemotherapy with chlorambucil has been replaced with targeted therapy with the small-molecule inhibitor of Bruton's tyrosine kinase (BTK), ibrutinib and monoclonal antibodies (mAbs) targeting CD20 (rituximab, obinutuzumab, and ofatumumab). Current standards of care for patients with relapsed or refractory CLL are ibrutinib, idelalisib alone or with rituximab, and venetoclax alone or with rituximab [18]. The developments of the last few years have been full of changes in an era of novel, molecularly targeted therapies, made possible by extensive efforts to illuminate the biology of the disease that predated the new targeted drugs. There is successful therapeutic targeting of the B-cell, such as Bruton's tyrosine kinase, phosphatidylinositol-3-kinase, and B-cell lymphoma. Moreover, new drugs targeted against CD37, myeloid cell leukemia 1 (MCL-1), cyclin-dependent kinase 9 (CDK9), colony-stimulating factor 1R (CSF1R), etc., hold promise for an even more robust therapeutic armamentarium in the near future [18]. Another attractive strategy for CLL therapy is to target proteins that control programmed cell death (apoptosis). Indeed, CLL is thought to result from an imbalance between proliferation and apoptosis. In addition, many factors are overexpressed in CLL lymphocytes such as proteins of the BCL-2 and IAP families. The BCL-2 family proteins control the mitochondrial outer membrane permeabilization (MOMP), responsible for the activation of caspases [19].

4 NATURAL COMPOUNDS FOR CLL TREATMENT

The ancient medical literature reports that natural products, especially plant products, have been used for a long time. Natural products have a relevant role in cancer therapy today with substantial numbers of anticancer agents used in the clinic. During the last few years, natural product-based drug discovery is increasing based on new technologies, such as high-throughput screening and combinatorial synthesis. Etoposide, paclitaxel, irinotecan, and vincristine are famous examples of plant-derived compounds; l-asparaginase, doxorubicin, bleomycin, mitomycin C, and actinomycin D are drugs coming from microbial sources, and citarabine was a first drug originating from a marine source. All these agents are characterized by a variety of different mechanisms of action, including inhibition of topoisomerases I or II, alkylation of DNA, interference with tumor signal transduction, and interaction with microtubules [20,21].

Many natural compounds have been used in the past decades to treat several diseases due to their antidiabetic, hepatoprotective, and cardioprotective activities [22]. Recently, the scientific research has focused on natural

compounds and their metabolites with preventive and therapeutic activities, mainly phytochemicals such as terpenes, lignans, and polyphenolic compounds, as new potential chemopreventive agents with apoptotic effects on cancer, multidrug resistance reverting potential, or cytotoxic activity [23]. Moreover, combination therapy, that joins conventional chemotherapy with natural compounds, is now considered a new promising strategy to reduce cellular toxicity and overcome MDR, in particular in CLL due to its development of leukemogenesis and very complex origin [24]. In the following, we want to update and summarize the recent applications of natural compounds in the treatment of CLL.

4.1 Curcumin

Curcumin (Fig. 16.1) has efficient chemosensitizing, chemopreventive, chemotherapeutic, and antioxidant activities [25,26]. The apoptosis of CLL B-lymphocytes induced by curcumin is dose-dependent, and healthy B lymphocytes are less sensitive to its cytotoxic effects compared to CLL B-lymphocytes [27]. This compound induces PARP cleavage in primary CLL B-lymphocytes [2], that lead to the activation of programmed cell death [28]. Interestingly, the authors did not show activation of upstream caspases following curcumin exposure. In primary CLL B-lymphocytes obtained from CLL patients, the mechanism of PARP cleavage remains unclear. The release of cytochrome *c* from the mitochondrial membrane is another cellular event that contributes to curcumin-induced apoptosis [29].

In addition, curcumin also inhibits the constitutive activation of prosurvival pathways, some of which are preferentially active in primary CLL B-lymphocytes, including NF- κ B, Akt, and STAT3 [30]. STAT3 plays a significant role in the induction of angiogenic factors and antiapoptotic genes, and is vital to various cytokine signaling pathways [30]. Curcumin effectively inhibits constitutive STAT3 phosphorylation in CLL B-lymphocytes [30]. Mcl-1, a prosurvival gene downstream of STAT3, is also down-regulated by curcumin in primary CLL B-lymphocytes [31]. Constitutive phosphorylation of Akt is down-regulated by curcumin in primary B-CLL lymphocytes [32].

NF- κ B transcription factor is a prosurvival molecule that seems to be universally inhibited by curcumin. NF- κ B is down-regulated by curcumin in many different cancers.

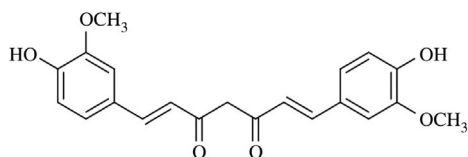


FIG. 16.1 Curcumin chemical structure.

Constitutive phosphorylation of I κ B α is inhibited by curcumin in primary CLL B-lymphocytes, indicating that genes downstream of NF- κ B should be inhibited in these cells. Following curcumin treatment, XIAP, a downstream target of NF- κ B, is down-regulated in primary CLL B-lymphocytes [33], an interesting finding considering previous study demonstrating that Bcl-2 is a direct transcriptional target of NF- κ B and is down-regulated by curcumin [32]. Treatment of CLL B-lymphocytes with curcumin up-regulates BIM, a proapoptotic protein. Hence, it can down-regulate survival pathways and up-regulate apoptotic pathways in CLL B-lymphocytes [34].

There are several limitations to the anticancer activity of curcumin, such as its poor absorption, poor pharmacokinetics, low bioavailability, low water solubility, slow cellular uptake, and rapid metabolism [35]. Bioavailability of curcumin can be increased by liposomal encapsulation and using nanoparticle formulations, increasing membrane permeability through the incorporation of it in micelles and phospholipids formulation, decreasing hydrophobicity and chemical modifications [36]. Also, the bioavailability of curcumin can also be attempted through intravenous infusions. Moreover, quercetin and prednisone can enhance the action of curcumin [34].

4.2 Quercetin

Quercetin (3,3',4',5,7-penta-hydroxyflavone) (Fig. 16.2) belongs to the wide group of natural anticancer compounds proposed as apoptotic inducers in chemotherapy or in adjuvant chemotherapy when associated with other drugs. The role of quercetin as an anticancer agent has been studied by several researchers. Gokbulut et al. demonstrated that treatment of CLL B-lymphocytes with quercetin and resveratrol caused dose-dependent inhibition of cell proliferation and increased apoptotic cell population through induction of caspase-3 activity. Cell cycle analysis displayed cell cycle arrest mainly in G₀/G₁ for both polyphenols [37]. Russo and colleagues have shown that quercetin significantly enhances anti-CD95- and rTRAIL-induced cell death, increases caspase-3 and -9 activities, and positivity to Annexin V. In addition, they proved that association of quercetin with fludarabine increases the apoptotic response in CLL B-lymphocytes of about two-fold, compared with quercetin monotreatment [38].

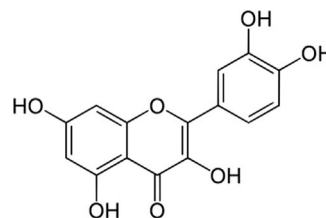


FIG. 16.2 Quercetin chemical structure.

In another study Russo and colleagues confirmed that quercetin in association with ABT-737 synergistically enhances apoptosis in CLL B-lymphocytes. They also reported that the cellular uptake of quercetin is extremely rapid, with an intracellular concentration of about $38.5 \text{ ng}/10^6$ cells, after treatment with $25 \mu\text{M}$ for 5 min. They demonstrated that the activity of protein kinase CK2, which positively triggers PI3K/Akt pathway by inactivating PTEN phosphatase, is inhibited by quercetin immediately after its addition to CLL B-lymphocytes (0–2 min). PI3K activity was also inhibited by quercetin within 60 min from the treatment. The combined inhibition of CK2 and PI3K kinase activities by quercetin restored ABT-737 sensitivity and increased lethality in human leukemia cells [39].

Quercetin gallate tea-derived flavonol is also a multi-target apoptosis inducer and proteasome inhibitor [40]. This compound can trigger both the intrinsic and extrinsic pathways in primary CLL B-lymphocytes, notably by Mcl-1 down-regulation; the mechanism involves inhibition of the PI3K/AKT pathway, which in turn leads to the instability of Mcl-1 mRNA and protein [38].

4.3 Resveratrol

Resveratrol (Fig. 16.3) is a phytoalexin from the stilbene family of polyphenols present in numerous fruits, plants, and vine products. Resveratrol was already known to protect against cardiovascular problems. Studies have revealed that this compound also displays potential anti-cancer properties. The polyphenol compounds regulate many different molecular targets and signaling pathways such as ROS production, p53, AP-1, members of the IAP and Bcl-2 families, proteasome activity, NF- κ B, PI3K/AKT, MAP kinase, TRAIL-death receptor, and mitochondrial pathways [41]. Resveratrol therefore displays a synergistic effect in association with other agents for treatment of CLL [37]. It is reported that resveratrol induces apoptosis in primary CLL B-lymphocytes, triggers the mitochondrial pathway, and activates caspase-3 [42].

4.4 Epigallocatechin Gallate

Epigallocatechin gallate (EGCG) (Fig. 16.4), the principal polyphenol in green tea, induces apoptosis in CLL B-lymphocytes in vitro by caspase-3 activation and PARP

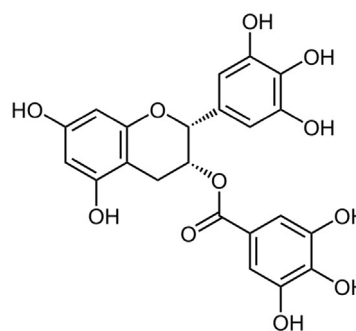


FIG. 16.4 Epigallocatechin gallate chemical structure.

cleavage and also through partial inhibition of VEGFR1 and VEGFR2 phosphorylation. Bcl-2 is down-regulated by EGCG, as is Mcl-1 and XIAP [43]. Treatment of primary CLL B-lymphocytes with the combination of curcumin and EGCG was researched. EGCG has pleiotropic effects. It can notably trigger caspase-dependent mitochondrial apoptosis in many tumor models, including primary CLL B-lymphocytes in which it down-regulates XIAP and Mcl-1 [44]. Apoptotic pathways such as proteasome inhibition are involved in EGCG's mechanisms of action. A Phase II study of EGCG in CLL has shown promising results with reduction in lymphocytosis and/or adenopathy in 29 out of 42 patients (69%) [45].

4.5 Apigenin

Apigenin (Fig. 16.5) is a common dietary flavonoid found in many vegetables, Chinese medicinal herbs, and fruits and has multiple physiological functions, such as antiviral, antibacterial, antioxidant, and strong anti-inflammatory activities and blood pressure reduction [46]. Recently, apigenin has been widely researched for its anti-tumor activities and low toxicity. Apigenin was observed to suppress various human cancers in vitro and in vivo by multiple biological effects, such as inducing cell cycle arrest triggering, cell apoptosis and autophagy, invasion and suppressing cell migration, and stimulating an immune response. There are several signaling pathways modulated by apigenin, including the PI3K/AKT, MAPK/ERK, JAK/STAT, NF- κ B and Wnt/ β -catenin pathways [47]. Apigenin has proapoptotic activity in B-CLL lymphocytes, and this effect has been associated with inhibition of the PI3K/AKT pathway and decreased AKT phosphorylation. Also, apigenin is known to inhibit

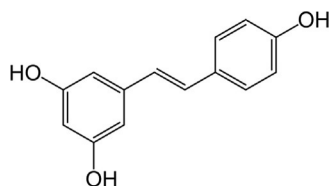


FIG. 16.3 Resveratrol chemical structure.

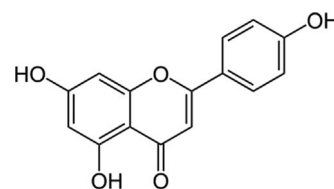


FIG. 16.5 Apigenin chemical structure.

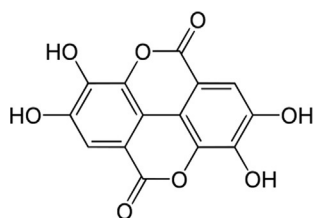


FIG. 16.6 Ellagic acid chemical structure.

both proteasome and CDK activities [40] and to target several apoptotic pathways; the compound may act through several mechanisms in CLL B-lymphocytes [48].

4.6 Ellagic Acid

Ellagic acid (EA) (Fig. 16.6) is a natural phenolic constituent that is contained in ellagitannins in grapes, strawberries, nuts, raspberries, black currents, pomegranates, green tea, and the bark and stem of *Eucalyptus maculata*, *Eucalyptus globulus*, and nuts. Recent in vitro and in vivo experiments have revealed that EA elicits anticarcinogenic effects by inhibiting tumor cell proliferation, breaking DNA binding to carcinogens, blocking virus infection, disturbing inflammation, inducing apoptosis and angiogenesis, and drug-resistance processes required for tumor growth and metastasis [49,50]. Based on our study EA decreases the percentage of viable cells and

induced apoptosis also increased ROS formation, mitochondria swelling, MMP decrease, and cytochrome *c* release in mitochondria isolated from CLL B-lymphocytes (Fig. 16.7). Our results suggest that EA can act as an anticancer candidate by directly and selectively targeting mitochondria that could induce apoptosis through the mitochondria pathway with increasing ROS production, which finally ends in cytochrome *c* release, caspase 3 activation, and apoptosis in cancerous B-lymphocytes isolated from CLL patients [51].

4.7 Acacetin

Natural agents are employed in cancer chemoprevention to reverse, suppress, or prevent cancer progression. Acacetin (5,7-dihydroxy-4'-methoxyflavone) (Fig. 16.8) exerts antiparasitodal, antimutagenic, antiinflammatory, antiperoxidant, and anticancer effects by suppressing the migration and invasion of human cancer cells. Acacetin has also been shown to exert an antiproliferative effect by blocking cell cycle progression and inducing apoptosis [52]. Salimi et al. have found that acacetin (10 μ M) can selectively induce apoptosis on CLL B-lymphocyte (25% at 24h) by directly targeting mitochondria, through increased reactive oxygen species (ROS) formation, MMP collapse, MPT, release of cytochrome *c*, caspase 3 activation, and finally apoptosis, while sparing normal healthy B-lymphocytes unaffected at similar concentrations

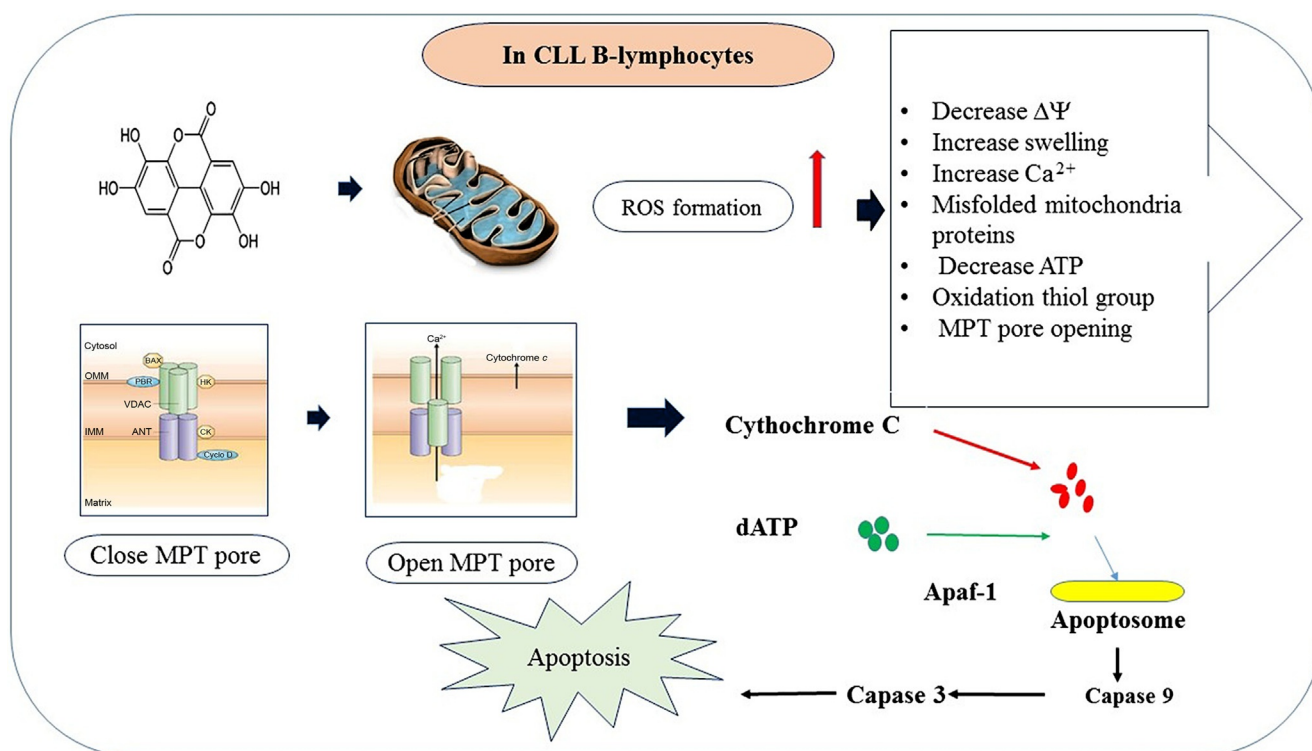


FIG. 16.7 The mechanism of action of ellagic acid described by Salimi et al. on CLL B-lymphocytes [51].

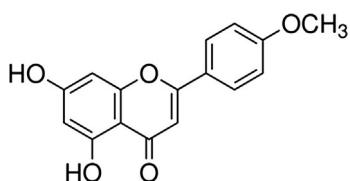


FIG. 16.8 Acacetin chemical structure.

(Fig. 16.9). In addition, oral administration of acacetin showed a potent in vivo anticancer activity in CLL xenograft mouse models. These findings indicate that acacetin accumulates and kills CLL B-lymphocyte in a rather selective way through targeting cancerous mitochondria and ROS formation, which ends in CLL therapy [53].

4.8 Chrysin

Chrysin (5,7-dihydroxy-2-phenyl-4H-chromen-4-one) (Fig. 16.10) is a natural flavonoid. Chrysin has been recently shown to be a potent inhibitor of aromatase and of human immunodeficiency virus activation in models of latent infection. It has also demonstrated antioxidant and antiinflammatory effects, and has shown cancer chemopreventive activity via induction of apoptosis in a diverse range of human and rat cell types. In most of the cancer cells, chrysin has been shown to induce apoptosis and inhibit proliferation, and is more potent than other tested flavonoids in leukemia cells, where chrysin is likely to act via activation of caspases and inactivation of Akt signaling in the cells. Moreover, structure-activity relationships have revealed that the chemical structure of chrysin meets the key structural requirements of

flavonoids for potent cytotoxicity in leukemia cells [54]. Zaric and colleagues demonstrated that exposure of MOLT-4, JVM-13 cell lines and B-CLL cells to the concentration of chrysin of 10 μ M and higher selectively decreased viability of cells in this cell population, but not in the PBMC derived from healthy subjects; LC50 values of chrysin for B-CLL cells were 51 μ M for 24h and 32 μ M for 48h of incubation, respectively. These findings demonstrated that chrysin induces the activation of proapoptotic Bax and decreases the expression of antiapoptotic Bcl-2 protein, releases cytochrome *c* from the mitochondria into cytosol, and cleavages/activates caspase-3, subsequently leading to the activation of apoptosis of B-CLL cells [55]. Also, Salimi et al. investigated the effect of chrysin on isolated normal and CLL B-lymphocytes and their isolated mitochondria. They reported a selective and significant increase in cytotoxicity, intracellular reactive oxygen species, mitochondrial membrane potential collapse, ADP/ATP ratio, caspase 3 activation, and finally apoptosis in chrysin-treated CLL B-lymphocytes. Moreover, they determined that chrysin selectively inhibits complex II and ATPases in cancerous mitochondria [56].

4.9 Wogonin

Wogonin (5,7-dihydroxy-8-methoxy-2-phenyl-4H-chromen-4-one) (Fig. 16.11) is a traditional Chinese herb obtained from *Scutellaria baicalinase's Georgi* that has been widely researched and used for inflammatory and allergic diseases with its medication effects of potential removing toxins and cleansing heat. Studies have shown inhibitory effects of wogonin on a number of different

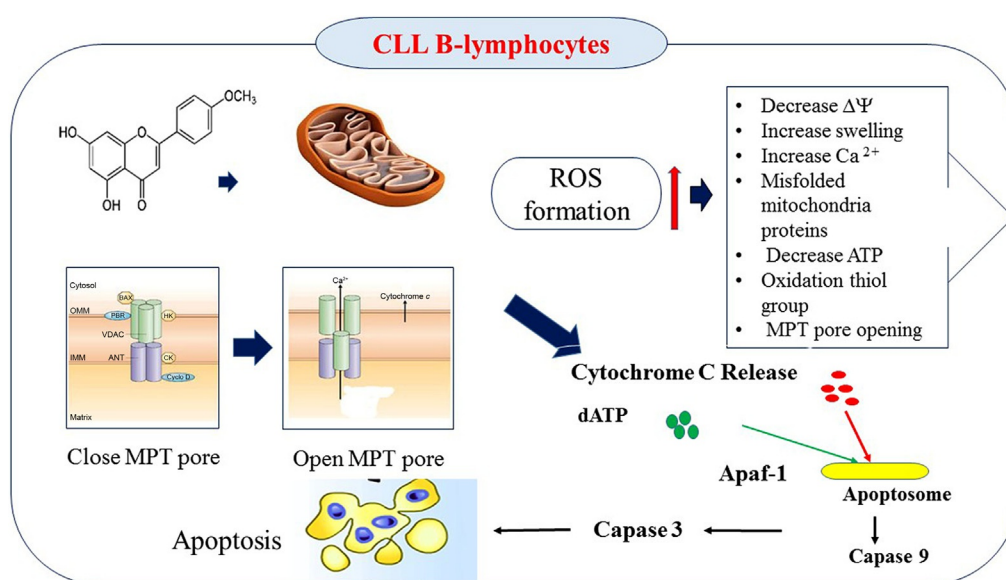


FIG. 16.9 The mechanism of action of acacetin described by Salimi et al. on CLL B-lymphocytes [53].

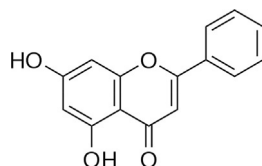


FIG. 16.10 Chrysin chemical structure.

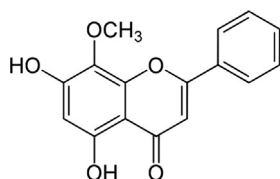


FIG. 16.11 Wogonin chemical structure.

cancer cells. The mechanisms of action are modulation of the p53 signaling pathway, G1 phase arrest, antitumor angiogenesis by inhibition of VEGF, and apoptosis through the mitochondrial pathway. These works indicate that wogonin is a new anticancer agent with enhancement of the curative effect on chemo-insensitive tumors that may clinically benefit ovarian cancer patients [57]. Polier et al. demonstrate for the first time that wogonin and structurally related natural flavones, for example apigenin, chrysin, and luteolin, are inhibitors of cyclin-dependent kinase 9 (CDK9) and block phosphorylation of the carboxy-terminal domain of RNA polymerase II. This effect leads to reduced RNA synthesis and subsequently rapid down-regulation of the short-lived antiapoptotic protein myeloid cell leukemia 1 (Mcl-1) resulting in apoptosis induction in cancer cells. They showed that genetic inhibition of Mcl-1 or CDK9 expression by siRNA is sufficient to mimic flavone-induced apoptosis. Pull-down and in silico docking studies demonstrate that wogonin directly binds to CDK9, presumably to the ATP-binding pocket [58]. Dürr et al. showed in CLL cocultures that wogonin treatment results in a concentration-dependent apoptosis induction, which is significantly increased by the addition of TNF α . Also, they proved that wogonin significantly reduces spleen weights and leads to a reduced CLL content in the spleen, the bone marrow, and the peritoneal cavity. If treatment is started in the advanced disease stage, wogonin slightly lowered spleen weight and the CLL content in the spleen, whereas the percentage of CLL B-lymphocytes in the peripheral blood was increased. Interestingly, in their work wogonin treatment results in a loss of cell surface TNFR1 expression in splenic CLL B-lymphocytes and increased TNFR1 levels in the serum. These data suggest that wogonin induces a redistribution of CLL B-lymphocytes in vivo, preventing their homing to lymphoid organs and that loss of TNFR1 expression might be involved in this process [59].

5 CONCLUSIONS

With a rising number of new cases of CLL, novel agents that could cure or prevent cancers are still very much needed. Most anticancer agents act by induction of apoptosis and cell cycle arrest, as well as through an inhibition and proliferation of cell growth. This chapter indicates that many of the natural products have been shown to have antileukemic activities in CLL B-lymphocytes and that they exert their effects through one of the aforementioned mechanisms. It is expected that the effects of these compounds on cell cycle, cell growth, and proliferation, as well as induction of apoptosis in CLL B-lymphocytes, will provide clues for the prediction of novel agents that may be useful in cancer chemoprevention or chemotherapy. Also, the combination of some natural compounds enhances the antileukemic activity of some agents, while other reports indicate that such combinations are not always beneficial in terms of potentiating the action of other antileukemic agents. In addition, the chapter highlights some natural products obtained from plants and fruits that are used as medicines against CLL. Studies suggested that these agents show a good potential for combating CLL B-lymphocytes. These herbs and fruits could be the best candidates for future CLL therapy, with better acceptability as compared to chemotherapy, easier availability, and minimal adverse effects; it is also likely they will provide more potent antileukemic agents in future.

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